flight muscle fibers from *Drosophila melanogaster* indicate faster cross-bridge cycling kinetics during lengthening and slower cycling during shortening, compared to isometric contraction. During isometric contraction we estimate a myosin attachment time of 5.0 ms, and 4.6 versus 5.5 ms during the lengthening and shortening transients, respectively. These initial applications of the white-noise system analysis technique show promise for future studies probing molecular processes that underlie complicated length transients associated with normal muscle contraction.

1804-Pos

The Isotonic Velocity Transient Following a Sudden Rise in Force Imposed on the Muscle Sarcomere During Unloaded Shortening Reveals a Rate Limiting Step in Detached Myosin Motors

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Energy balance studies indicate that ATP splitting by myosin motors during rapid shortening of skeletal muscle is not sufficient to account for the energy (mostly heat) output (Rall et al., *J Gen Physiol* 68(1), 13, 1976; Homsher et al., *J Physiol* 321, 423, 1981). We investigated the kinetic step of the myosin ATP-ase cycle related to this phenomenon in single muscle fibers from *Rana esculenta* (~2.15 μm sarcomere length, 4°C), by recording the isotonic velocity transient following a force step from zero to the isometric tetanic value (T_0). Once the isometric tetanus had developed, the force was first clamped to zero for a range of times from 10 ms to 18 ms (during which the fiber shortened at the maximum velocity by 30 nm hs^{-1} to 50 nm hs^{-1}) and then raised again to T_0 in a stepwise manner (~120 μs). The elastic lengthening induced by the force step was followed by a transient isotonic lengthening, the size of which ranged from 40 to 60 nm hs^{-1} depending on the size of the preceding shortening. The lengthening velocity was larger for larger shortening size and progressively decreased to approach the isometric condition with a half-time of 2-3 ms. Similarly, the half-sarcomere stiffness recovered the isometric value e_0 from the unloaded shortening value of 0.4 e_0 with an exponential time course with τ ~3 ms. We conclude that during rapid shortening a ~3 ms-transition between detached states of the myosin motor, likely related to the ATP hydrolysis, becomes rate limiting. Accumulation of motors in the state preceding the hydrolysis step can account for the unexplained energy during rapid shortening. Supported by MIUR (Italy).

1805-Pos

Effect of Inorganic Phosphate on the Rate of ADP Release During Ramp Shortening in Activated Permeabilized Fibers from Rabbit Psoas Muscle

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A coumarin-labeled recombinant phosphorylated nucleoside diphosphate kinase (P~NDPK-IDCC; West et al., 2009; Biophys.J. 96:3281-3294), was used as a fluorescence probe for time-resolved measurement of changes in [MgADP] during steady shortening of single permeabilized rabbit psoas fibers at 12°C (pCa 4.5, pH 7.1, ATP 5.7mM). A fiber contracted from the relaxed state by immersion into a Ca^{2+} activation solution at 0°C. Temperature activation was then initiated by immersion of the fiber into silicone oil at 12°C. The activation solutions were prepared with either zero added P_i or with 10 mM added P_i at constant ionic strength (0.15 M). The decline in fluorescence intensity emission (470nm) associated with MgADP-dependent dephosphorylation of P~NDPK-IDCC (60 μM) was monitored during activation and during a period of isovelocity shortening. Fluorescence emission (580 nm) of a rhodamine dye was measured simultaneously to correct the P~NDPK-IDCC signal for the effects of fiber movements and volume changes. The rate of MgADP release in the absence of added P_i increased from 0.7 ± 0.07 mM·s^{-1} at 0.2 fiber-lengths·s^{-1} to approximately 3.4 ± 0.25 mM·s^{-1} for shortening velocities between 1 and 2 fiber-lengths·s^{-1}. When 10 mM P_i was added, the rate of ADP release at 0.2 fiber-lengths·s^{-1} was 0.48 ± 0.05 mM·s^{-1} and 2.6 ± 0.4 mM·s^{-1} at 1-2 fiber-lengths·s^{-1}. In the absence of added Pi, the rate of ATP hydrolysis calculated from the appearance of ADP is similar to that calculated previously from the appearance of P_i using MDCC-labeled phosphate binding protein, over the same range of fiber shortening speeds (He et al., 1999, J. Physiol. 517: 839-854). Thus P_i slows the ATPase rate by 25-30%, both in the isometric and isotonic state. The energetic consequences will be discussed.

1806-Pos

Cross-Bridges and Sarcomere Stiffness in Single Intact Frog Muscle Fibers

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The number of cross-bridges formed in activated skeletal muscles is a key information for both energetics and mechanics of muscle contraction. In this study we determined the cross-bridge number in single fibers by measuring the tension P_c which forcibly detached the cross-bridge by fast stretches (Bagni et al. 2005, *J. Physiol* 565). Fibers, isolated from the tibialis anterior muscle of *Rana esculenta*, were mounted between an electromagnetic motor and a fast force transducer. Sarcomere length was measured by means of a striation follower device. Measurements were made during tetanus rise in normal Ringer and in sub-maximal tetanic contractions in Ringer-BTS (*N*-benzyl-p-toluene sulphonamide, 1 μM) at 5°C at sarcomere length of 2.1 μm.
The results were compared with fiber stiffness, another indicator of cross-bridge number, measured with 4 kHz sinusoidal length oscillations (1 $nmhs^{-1}$ p-p amplitude). The stiffness-tension relation was the same both during the tetanus rise and Ringer-BTS and showed the non-linearity expected from the myofilament compliance. However, the data could not be fitted satisfactorily with a simple model made of cross-bridge and linear filament compliances in series. A good fit was obtained by assuming that a fraction (~14%) of attached bridges at tetanus plateau was generating no-force. Relative myofilament and cross-bridge compliance resulted 0.37 and 0.63 respectively. The stretch data showed a linear relation between P_c and tension with a slope consistent with the presence of the non-force generating bridges suggested by stiffness data. These results suggests the existence of a possible non-linearity between cross-bridge force and stiffness and show that the relation between fiber stiffness and cross-bridge number is not as simple as usually assumed.

1807-Pos

Ultrafine Striations in Skeletal Muscle Revealed by 3D Super-Resolution Fluorescence Microscopy

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Super-resolution three-dimensional imaging was achieved using newly synthesized photoactivatable quantum dot probes. Semiconductor quantum dots are nanoparticles with high photostability and brightness. They were modified with a novel quencher system to make them photoactivatable. The unique properties of these photoactivatable quantum dots enable single-fluorophore localization in three dimensions using a confocal microscopy optical sectioning method with a piezo scanner. To image skeletal muscle at resolutions exceeding that of the standard confocal microscope, the photoactivatable quantum dots were conjugated to secondary antibodies. Myofibrilar bundles were dual- labeled using both a primary antibody to myosin rod with the secondary antibody-conjugated photoactivatable 655 nm quantum dot and a primary antibody against tropomyosin with the secondary antibody-conjugated photoactivatable 525 nm quantum dot. During the 3D acquisition on a spinning disk confocal with piezo scanner, different individual quantum dots were photoactivated during each of hundreds of cycles. A sufficient number of single quantum dots were localized, reduced to their center of mass and then reconstructed to a super-resolution image. The resulting super-resolution image shows a sub-diffraction resolution in both lateral and axial directions. The broad absorbance band of quantum dots enables the excitation of both quantum dots with the same laser type. This technique enables the relative localization of two different myofibril proteins at nanometer scale resolutions in solution demonstrates ultrafine striations in the staining pattern with widths less than 70 nm in axial and lateral dimensions that are not evident by conventional confocal microscopy due to its resolution constraints. The bands appear to be related to the presentation of epitopes at the surface of thin and thick filaments and may be related to thick and thin filament binding proteins and/or structural variations in the actin and myosin filaments.

1808-Pos

Structural and Functional Gradients with Temperature in the Flight Muscle of Manduca Sexta

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The force/extension curve of the flight muscle of the Hawkmoth, *Manduca sexta* is remarkably similar to that of mammalian cardiac muscle suggesting that it may serve as a useful model system for certain aspects of cardiac muscle structure and function (J Exp Biol. 2004;207:2455). More recently, it was discovered that these animals maintain an astonishing thermal gradient of 8.8 C in the 5 mm distance dorsal to ventral in their dorso-longitudinal flight muscles (DLMs). Does the existence of this thermal gradient necessarily imply a functional gradient? Do these changes in function have, as their basis, changes in structure? Twitch dynamics of individual fibers within the DLM in intact animals are temperature dependent so that mechanical power output (and it's phase dependence) varies with depth in the tissue. A surprising observation was that all five sub-units in the DLM were simultaneously activated. Cooler